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Afforestation with an age-sequence of Mongolian pine plantation promotes soil microbial residue accumulation in the Horqin Sandy Land, China

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Abstract: Land use change affects the balance of organic carbon (C) reserves and the global C cycle. Microbial residues are essential constituents of stable soil organic C (SOC). However, it remains unclear how microbial residue changes over time following afforestation. In this study, 16-, 23-, 52-, and 62-year-old Mongolian pine stands and 16-year-old cropland were studied in the Horqin Sandy Land, China. We analyzed changes in SOC, amino sugar content, and microbial parameters to assess how microbial communities influence soil C transformation and preservation. The results showed that SOC storage increased with stand age in the early stage of afforestation but remained unchanged at about 1.27−1.29 kg/m^2 after 52 a. Moreover, there were consistent increases in amino sugars and microbial residues with increasing stand age. As stand age increased from 16 to 62 a, soil pH decreased from 6.84 to 5.71, and the concentration of total amino sugars increased from 178.53 to 509.99 mg/kg. A significant negative correlation between soil pH and the concentration of specific and total amino sugars was observed, indicating that the effects of soil acidification promote amino sugar stabilization during afforestation. In contrast to the Mongolian pine plantation of the same age, the cropland accumulated more SOC and microbial residues because of fertilizer application. Across Mongolian pine plantation with different ages, there was no significant change in calculated contribution of bacterial or fungal residues to SOC, suggesting that fungi were consistently the dominant contributors to SOC with increasing time. Our results indicate that afforestation in the Horqin Sandy Land promotes efficient microbial growth and residue accumulation in SOC stocks and has a consistent positive impact on SOC persistence.

Keywords: soil organic matter; stand age; biomarker; amino sugars; microbial residues

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1 Introduction

Soil organic matter holds more organic carbon (C) than all of the global plants and atmosphere in combination, thus providing a critical ecosystem service (Lehmann and Kleber, 2015; Carvalho et al., 2022). Microbial activities are critical in soil organic C (SOC) pool formation because C dynamics in soils are typically the result of microbial catabolism and anabolism (Gougoulias et al., 2014). Microbial residues can be preferentially retained in soils by the growth of soil microbial

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biomass and the stability related to soil minerals (Miltner et al., 2012). It is critical to explore microbial residue accumulation and the associated process to understand the formation and stability of organic matter (Chen et al., 2020). Microbial residues (containing bacterial residues and fungal residues) comprise non-biomass microbial metabolites such as exo-enzymes, extracellular polymeric substances, and dead cell remains (Joergensen, 2018). The ratio of fungal residual C to bacterial residual C represents the relative contribution of fungi and bacteria to SOC. Microbial residues can be quantified using amino sugars because they are vital components of microbial cell walls, and they are absent in vegetation and relatively stable after microbial cell death (Simpson et al., 2007). Evaluating amino sugar dynamics can assist in determining the preservation of microbial residual C and the perpetual fluctuation in the composition of organic C (Amelung et al., 2002).

Land use change is an important driver for the balance of organic C stocks and the global C cycle (Poeplau et al., 2011). Previous research has shown that SOC is considerably influenced by changes in land cover (Bouchoms et al., 2017). For example, Poeplau and Don (2013) found that SOC stocks increased by 21 (±13) Mg/hm^2 after the conversion of cropland to forest land in northern Germany. Soleimani et al. (2019) observed that different land use patterns significantly influenced soil characteristics, and SOC decreased by 53% when a natural forest land was converted to an agricultural land in northern Iran. However, it remains unclear how the transformation of sandy land to agricultural or forest land drives amino sugars and microbial residues and their contribution to SOC. In recent years, most studies have focused on cropland, and only a few studies have investigated the influence of land use change on amino sugars or microbial residues. For example, Murugan et al. (2014) observed that the maize monoculture treatment reduced SOC storage and microbial residues compared with permanent grassland in Germany. Ding et al. (2020) found that the ratio of fungal to bacterial residue C decreased following the transformation of native grassland into cropland in northeastern China. However, detailed studies of microbial residue dynamics and their potential feedback to forest management in plantation ecosystems are scarce. The changes in the contributions of fungi and bacteria to SOC in plantations of different ages are unclear.

In the arid and semi-arid areas of northern China, including a sizable sandy area, afforestation became an important technique for land improvement (Hu et al., 2008). Horqin Sandy Land, located in the northeastern China, is a typical ecologically fragile area due to the long-term overgrazing and land reclamation (Liu et al., 2015). On primary sandy land, the distribution of sand is relatively uniform and there is almost no vegetation on the surface, leading them to become one of the most serious areas of desertification (Chang et al., 2003). Pine plantation is facilitated for the ability to serve as a net C sink because pine plants have high productivity but low forestation cost (Chapela et al., 2001). Mongolian pine (*Pinus sylvestris* var. *mongholica* Litv.) naturally grows in the Da Hinggan Ling Mountains and sand plains of Hulunbuir grassland (Song et al., 2016a, b). Because of its cold and drought resistance, Mongolian pine was initially introduced to the Horqin Sandy Land in the 1950s and quickly proved to be beneficial. This tree species has become a leading contender for the afforestation of sandy and semi-arid areas (Zhu et al., 2008). Accordingly, a massive amount of Mongolian pine has been planted in the northern areas of China over time, producing stands of different ages. These plantations act as windbreaks, fix sand, and conserve soil and water. However, as the age of Mongolian pine forests increases, it is unknown how the amino sugar and microbial residue accumulation will change and what the relative contributions of fungi and bacteria to SOC will be. Analyzing the changes in amino sugars and microbial residues, as well as their contributions during land use change, can provide insights for scientifically assessing the impact of afforestation on soil quality and carbon sequestration potential. The aims of this study were as follows: (1) to examine whether SOC accumulation was positive following afforestation; (2) to investigate the changes of amino sugars and microbial residues in cropland and Mongolian pine stands of different ages; and (3) to evaluate the relative contribution of fungi and bacteria to SOC after afforestation.

2 Materials and methods

2.1 Study area and materials

The study area is located in Liaoning Province, southeastern Horqin Sandy Land, northeastern China (42°43′N, 122°29′E; 242 m a.s.l.). The climate in this area is classified as a typical temperate continental monsoon with an annual average temperature of 6.64°C and an average annual precipitation of 481.70 mm (Song et al., 2016b). The main soil is aeolian sandy soil, the terrain is relatively flat, and the soil is uniform. Before afforestation, this area was a typical sandy land with almost no vegetation. In 1953, planting experiments with Mongolian pine were started in this area. After afforestation in different years, artificial plantations of Mongolian pine of different ages were formed.

In April 2021, 16-, 23-, 52-, and 62-year-old Mongolian pine stands (hereafter MP16, MP23, MP52, and MP62) and a 16-year-old cropland (CL16) adjacent to MP16 were selected for this study. All treatments were established in the original sandy land, and MP16 and CL16 stands were planted in the same year. Each of the sample sites has a radius of less than 2 km. The initial planting density of Mongolian pine in all sites was approximately 1200 plants/hm^2. Due to continuous growth and competition among trees, some weak and dead trees were thinned. The surveyed tree densities in 2021 were 850, 775, 675, and 550 plants/hm^2 in MP16, MP23, MP52, and MP62, respectively. Over the past 7 a, none of these experimental sites were artificially disturbed. The dominant understory herbaceous species include *Digitaria sanguinalis* (L.) Scop., *Chloris virgata* Sw., *Setaria viridis* (L.) P. Beauv., *Artemisia scoparia* Waldst. & Kit., and *Axyris amaranthoides* L.

For the cropland, the crop system was corn (*Zea mays* L.). In the spring of each year, the cropland was tilled to a 20-cm depth before planting. During the entire growing season, the N-P_2O_5-K_2O compound fertilizer (EZhong Corp., Hubei, China) was applied by local farmers: 108 kg/hm^2 N, 108 kg/hm^2 P_2O_5, and 108 kg/hm^2 K_2O per year. A small amount of straw was returned to the cropland in autumn.

2.2 Soil sampling

In each land use pattern, five plots (10 m×10 m) were selected and each plot included two subplots (2 m×2 m). In April 2021, three soil cores were randomly sampled at the 0–10 cm layer using a hand auger (4 cm in diameter) in each subplot and homogenized to form a mixed sample. Sampled soils were rapidly transported to the laboratory and screened via a 2-mm sieve. Parts of the soil samples were freeze-dried and parts of the air-dried soil samples were utilized to measure SOC. In addition, we collected extra soil samples from 10 subplots to determine bulk density (BD). For each land use pattern, 10 standard trees were selected to measure tree height and diameter at breast height (DBH) using a Blume-Leiss altimeter and tape measure. Detailed information for the sampling plots is provided in Table 1.

Table 1 Stand characteristics of Mongolian pine plantations with different ages

Age (a)	Tree height (m)	DBH (cm)
16	3.79±0.07	7.47±0.29
23	6.01±0.18	13.24±0.35
52	10.64±0.24	24.38±1.01
62	12.43±0.19	25.75±0.90

Note: Mean±SE. DBH, diameter at breast height.

2.3 Analysis of soil properties

SOC was identified by dry combustion method using a Vario TOC Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany) (Geiger and Hardy, 1971). Soil pH was determined by the soil slurry method (McLean, 1983). After shaking air-dried soil in distilled

water (water:soil (v:m)=2.5:1.0) for 2 min, the slurry was settled and the supernatant was determined with an electrode. SOC storage (kg/m^2) was calculated using the following equation:

$$\text{SOC storage} = \left(Con \times \text{BD} \times L \right) / 100 \,, \tag{1}$$

where *Con* is the SOC concentration (g/kg); BD is the bulk density (g/cm^3); and L is the calculated layer thickness (10 cm).

2.4 Amino sugar extractions and related analyses

Soil amino sugars were extracted following the procedure outlined by Zhang and Amelung (1996) to identify fungal and bacterial residues. In brief, 10.0 mL of 6 M HCl was used to hydrolyze air-dried soil samples that contained around 0.3 mg of nitrogen for 8 h at 105°C. The slurry was filtered, dried, rinsed, and purified using KOH. Then, the filtrate was freeze-dried overnight. The remainder was dissolved with 5.0 mL methanol and dried with N_2. The lyophilized remainder was dissolved in 300.0 μL of derivational solution and heated at 75°C–80°C for 35 min. Acetic anhydride was added to the derivatives and heated at 75°C–80°C for 25 min after being cooled to room temperature. Then 1.5 mL of dichloromethane and 1.0 mL of 1 M HCl were added and the anhydride was eliminated using deionized water. The residual organic phase was then dried with N_2 at 45°C. After being re-dissolved in 200.0 μL of ethylacetate-hexane, the amino sugar derivatives were analyzed by Agilent 6890A Gas Chromatography (Agilent Technologies, Palo Alto, USA). Chemstation software (Agilent Technologies, Palo Alto, USA) was used to identify the peaks by contrasting sample retention times to benchmarks for amino sugar standards.

2.5 Microbial parameters and calculations

Total amino sugars were estimated by sum of glucosamine (GluN), galactosamine (GalN), muramic acid (MurN), and mannosamine (ManN). The ratios of GluN to MurN and of GluN to GalN were used to express the relative contributions of fungi and bacteria to SOC, respectively (Liang et al., 2007).

Microbial residues are better indicators of microbial contribution to soil C pools than standing biomass. Bacterial residual C (BRC) was calculated by multiplying the concentration of MurN by 45, a conversion factor for bacterial residues (Appuhn and Joergensen, 2006). We calculated fungal residual C (FRC) using the following equation according to the method described by Joergensen (2018):

$$\text{FRC} = \left(\frac{\text{GluN}}{179.17} - \frac{2 \times \text{MurN}}{251.23} \right) \times 179.17 \times 9 \,, \tag{2}$$

where FRC is the fungal residual carbon; 179.17 is the molecular weight of GluN; 251.23 is the molecular weight of MurN; and 9 is the conversion factor for the concentration of GluN to fungal residues with an estimated ratio of 1:2 between MurN and GluN (Engelking et al., 2007). The sum of FRC and BRC was calculated to represent the total MRC.

2.6 Statistical analyses

One way analysis of variance (ANOVA) was used to test the differences in land use patterns. The ANOVA analyses were conducted using SPSS v.24.0 (IBM Corp., Armonk, NY, USA). A Fisher's least significant difference (LSD) test was used to compare the means when the difference was significant (P<0.050).

3 Results

3.1 Soil properties

Table 2 shows that soil BD of cropland was significantly lower than that of MP16, indicating an obvious decrease in soil BD due to tillage. Results from Mongolian pine plantation with different ages revealed that soil BD remained almost unchanged after 16-, 23-, and 52-year-old of afforestation. However, soil BD decreased significantly after 62 a. Soil pH decreased gradually

with increasing stand age, indicating that soil acidification increased during afforestation.

In the first 16-year-old of afforestation, SOC concentration of Mongolian pine stand was lower than that of cropland (Table 2). Stand age had a significant effect on SOC concentrations after afforestation, which ranged from 3.14 to 8.25 g/kg. SOC concentration increased with increasing stand age, indicating the distinct accumulation process of organic C after afforestation. However, after afforestation for 52 a, SOC concentration did not increase significantly compared with MP62. SOC storage exhibited similar trends that remained nearly constant after 52 a.

Table 2 Soil bulk density (BD), pH, soil organic carbon (SOC) concentration, and SOC storage at the 0–10 cm soil layer

Land use	BD (g/cm^3)	pH	SOC concentration (g/kg)	SOC storage (kg/m^2)
CL16	1.51 ± 0.02^{b}	6.45 ± 0.04^{b}	4.75 ± 0.39^{b}	0.72 ± 0.06^{bc}
MP16	1.61 ± 0.01^{a}	6.84 ± 0.04^{a}	3.14 ± 0.04^{c}	0.50 ± 0.01^{c}
MP23	1.63 ± 0.01^{a}	6.76 ± 0.04^{a}	5.33 ± 0.64^{b}	0.86 ± 0.11^{b}
MP52	1.63 ± 0.02^{a}	5.85 ± 0.11^{c}	7.97 ± 0.74^{a}	1.29 ± 0.12^{a}
MP62	1.54 ± 0.04^{b}	5.71 ± 0.12^{c}	8.25 ± 0.23^{a}	1.27 ± 0.04^{a}

Note: Different lowercase letters within the same columns represent significant differences among different land use patterns at $P<0.050$ level. Mean±SE. CL16 is the 16-year-old cropland; MP16, MP23, MP52, and MP62 are the 16-, 23-, 52-, and 62-year-old Mongolian pine plantations, respectively.

3.2 Amino sugars

At the early stage, total amino sugars of CL16 were significantly higher than that of MP16 (Fig. 1). Similarly, the specific amino sugars, i.e., GluN, GalN, and MurN exhibited the same patterns. For Mongolian pine stands established in different years, the concentrations of total and specific amino sugars both increased as stand age increased. In particular, the total amino sugars of MP62 were 153.1% higher than MP16. GluN, GalN, and MurN concentrations in MP62 significantly increased by 179.1%, 146.2%, and 164.2%, respectively, compared with MP16.

There was no significant difference in the ratios of GluN/MurN and GluN/GalN between MP16 and CL16 (Fig. 2). Moreover, different stand ages had no significant effect on the ratios of GluN/MurN and GluN/GalN, indicating that the contribution of fungi to soil organic matter was consistently dominant.

3.3 Fungal and bacterial residual C

Microbial residues are considered major constituents of the relatively stable C pool in soils. Figure 3 shows total MRC, BRC, and FRC concentrations in CL16 were significantly higher than those of the Mongolian pine stand established at the same time. Moreover, the concentrations of MRC, BRC, and FRC were significantly affected by stand age (Fig. 3). For example, the concentrations of total MRC, BRC, and FRC in MP62 increased by 175.0%, 164.2%, and 181.8%, respectively, compared with MP16.

Figure 3 shows that total MRC accounted for 29.0%–4.70% of SOC in the five studied treatments. For Mongolian pine stands with different ages, stand age did not significantly affect the contributions of BRC, FRC, and total MRC/SOC ratio (Fig. 3d–f). In addition, the ratio of FRC/BRC was consistently higher than 1 in all treatments (Fig. 2).

3.4 Correlations between soil pH and amino sugars

Figure 4 shows a significant negative correlation ($P<0.001$) between soil pH and concentrations of specific and total amino sugars. The concentration of total amino sugars tended to increase as soil pH declined, ranging from 178.53 mg/kg with pH>6.76 to 509.99 mg/kg in soils with pH<5.85 (Fig. 4d).

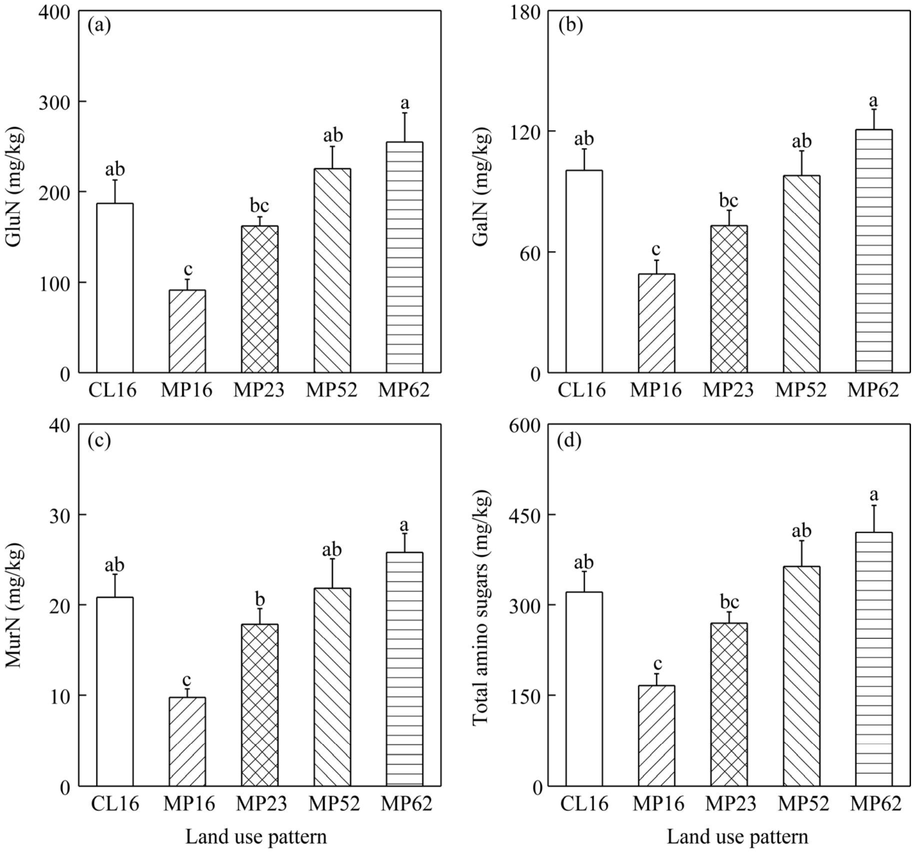

Fig. 1 Concentrations of specific and total amino sugars under different land use patterns. (a), GluN (glucosamine); (b), GalN (galactosamine); (c), MurN (muramic acid); (d), total amino sugars. CL16 is the 16-year-old cropland; MP16, MP23, MP52, and MP62 are the 16-, 23-, 52-, and 62-year-old Mongolian pine plantations, respectively. Bars mean standard errors. Different lowercase letters represent significant differences among different land use patterns at $P<0.050$ level. The abbreviations are the same in the following figures.

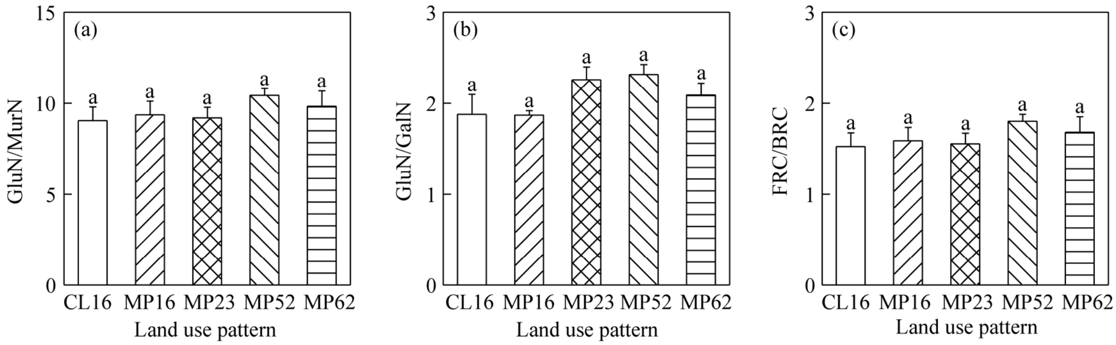

Fig. 2 Microbial parameters under different land use patterns. (a), GluN/MurN (ratio of glucosamine to muramic acid); (b), GluN/GalN (ratio of glucosamine to galactosamine); (c), FRC/BRC (ratio of fungal residual C to bacterial residual C). Different lowercase letters represent significant differences among different land use patterns at $P<0.050$ level.

4 Discussion

4.1 Effects of afforestation on soil BD, pH, and SOC

In the present study, the effect of afforestation on soil acidification caused soil pH to consistently decrease with increasing Mongolian pine stand age (Table 2). This result is consistent with

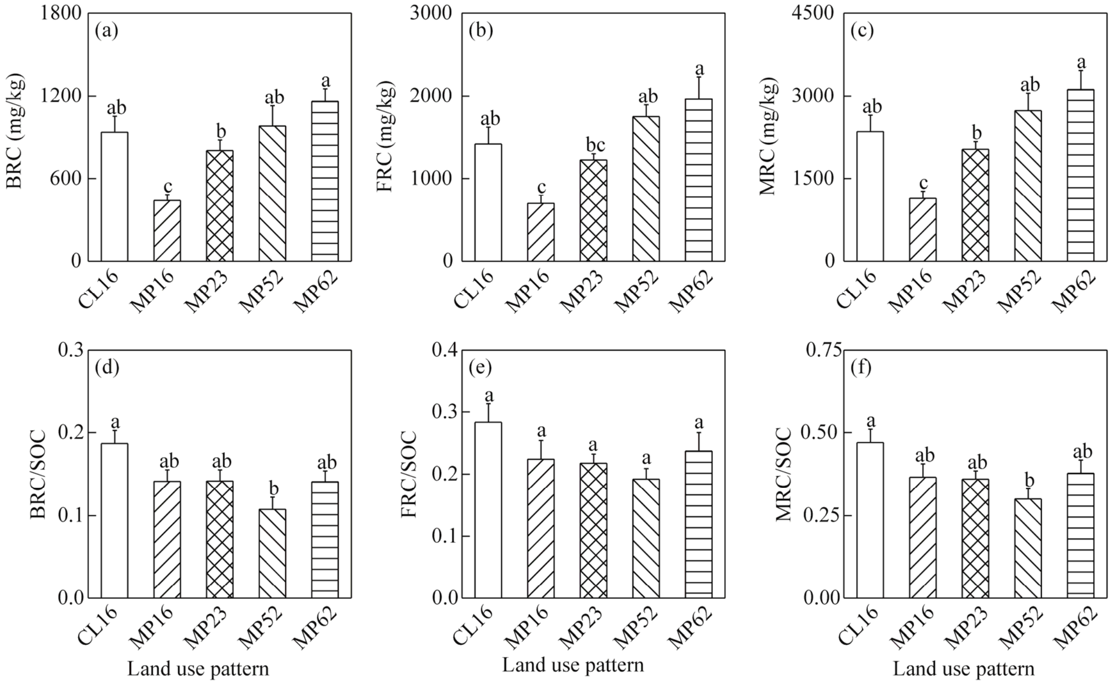

Fig. 3 Concentrations of microbial residual carbon (MRC) and their contributions to soil organic carbon (SOC) accumulation under different land use patterns. (a), BRC (bacterial residual carbon); (b), FRC (fungal residual carbon); (c), total MRC; (d), BRC/SOC; (e), FRC/SOC; (f), MRC/SOC. Different lowercase letters represent significant differences among different land use patterns at P<0.050 level.

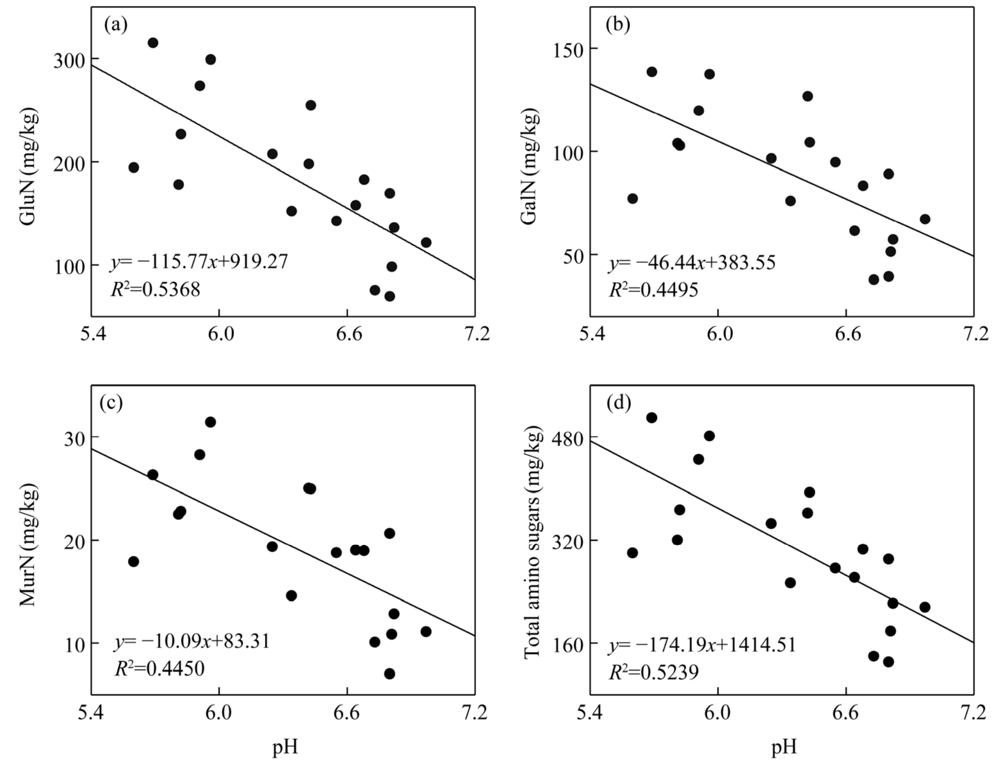

Fig. 4 Correlations between soil pH and concentrations of specific and total amino sugars. (a), GluN (glucosamine); (b), GalN (galactosamine); (c), MurN (muramic acid); (d), total amino sugars.

other studies (Berthrong, et al., 2009; Tew et al., 2021), which showed that afforestation lowered soil pH, especially under conifer forests. The considerable decrease of pH may be related to the redistribution of cations after afforestation, which increases the hydrogen ions in soil because of cation capture by roots or acid precipitation (Jobbágy and Jackson, 2003). Tree root exudates and rhizosphere environment changes may also result in a decrease in soil pH (Hinsinger et al., 2003). Moreover, the increase in acidity with increasing stand age can be attributed to the accumulation of acidic SOC (Mueller et al., 2012).

Table 2 shows that soil BD of cropland was significantly lower than that of Mongolian pine stand of the same age, which was caused by cultivation activities and tillage (Kocyigit and Demirci, 2012). With the exception of MP62, similar soil BD was observed for the plantations, which indicates that the change in BD is minimal with increasing age if there is no artificial interference on soil. A decreased soil BD was only observed in MP62, which may be because the amount of roots and litter increased after 62-year-old of afforestation (Zeng et al., 2014; Rytter, 2016).

The equilibrium of two biotic processes—the productivity of terrestrial plants and the decomposition of organic matter—determines SOC content. The amount of SOC produced by decomposition of vegetation litterfall is largely determined by root turnover and root exudates (Post et al., 1996). In this study, SOC concentration in MP16 was lower than that in CL16 (Table 2), supporting similar results reported by other studies (Poeplau et al., 2011; Lei et al., 2019). In contrast to CL16, a low SOC concentration of MP16 may be the reason that trees at the early growth stage absorb an enormous quantity of soil nutrients (Perron et al., 2021). Moreover, there was limited above-ground C intake because of the low rate of litterfall and low forest biomass (Hu et al., 2008), whereas fertilizer application increased SOC concentration of cropland (Manna et al., 2007).

For afforestation of sandy land, SOC concentration also increased with increasing stand age (Table 2), indicating that artificial afforestation is an effective approach to C sequestration, which was consistent with the results of Zhang et al. (2019) and Shao et al. (2019). The litter returned to the soil increased with increasing afforestation time, which contributed to SOC accumulation (Mondini et al., 2006). Moreover, the expanding plant cover and growing root systems as stand age increases may promote aggregate formation and reduce soil organic matter loss (Sauer et al., 2012).

Table 2 also shows that SOC storage remained almost constant after 52-year-old of afforestation, which suggests that Mongolian pine stand at this stage may have reached C saturation, making it difficult to further accumulate C. This result is consistent with previous reports that each mineral matrix has a finite capacity to stabilize organic matter (Bárcena et al., 2014; Shao et al., 2019; Hoover and Smith, 2023). The stabilization of organic materials by soil matrix is influenced by factors such as mineral surfaces, multivalent cations, architecture of soil matrix, and chemical nature of soil mineral fractions. Each mineral soil has a unique and finite capacity to stabilize organic matter, resulting in a saturation limit for the whole soil C (Baldock and Skjemstad, 2000). Moreover, this phenomenon may also be related to the decline of photosynthetic capacity and plant productivity in mature and over-mature Mongolian pine plantations (Wright et al., 2005; Liu et al., 2018).

4.2 Microbial parameters as indicators of SOC dynamics

Amino sugars are stable and heterogeneous in soil, and can be used to indicate the microbial response to afforestation (Glaser et al., 2004). In this study, the concentrations of GluN, GalN, and MurN all increased significantly with increasing afforestation age. This finding is consistent with a previous report that suggests amino sugars increase with forest age in broad-leaved Korean pine mixed forests (Shao et al., 2019). The proactive assimilation of C from vegetation into the microbial biomass, which is further enhanced by turnover and preservation of assimilating products underground, may result in increased retention of amino sugars after afforestation (Schimel et al., 2007; Cornwell et al., 2008; Liang et al., 2017). Moreover, three specific and total

amino sugar concentrations in CL16 were significantly higher than those in MP16 (Fig. 1). This may be due to the addition of fertilizers into cropland, which provides organic C and inorganic nitrogen sources for microbial growth and promotes the proliferation of microorganisms, thus substantially increasing the amino sugar concentrations of cropland (Roberts et al., 2007).

In addition, amino sugars are also influenced by soil pH. A negative correlation between pH and amino sugars was observed in this study (Fig. 4), which suggests that conditions of low soil pH may stimulate the decomposition of complex polymers to amino sugars, particularly chitin (Hu et al., 2018). As pH decreases, amino sugar stabilization may become more effective, particularly when amino sugars are combined with clay minerals to form persistent organo-mineral complexes (Sokol et al., 2019). Furthermore, low soil pH may increase the fungal growth rate, resulting in the accumulation of amino sugars (Rousk et al., 2009).

The ratios of GluN/MurN and GluN/GalN were used to represent the relative contribution of fungi and bacteria to soil organic matter, wherein higher ratio values indicate a more important contribution of fungi to soil organic matter (Liang et al., 2007). Figure 2 showed that either the reclaimed cropland or afforestation on previous sandy land of various ages did not significantly influence the values of GluN/MurN and GluN/GalN. This is probably because the duration of afforestation in the present research was insufficient to disrupt the protection mechanisms of SOC and influenced the structure of microbial community (Weedon et al., 2012).

In all treatments, the GluN/MurN ratio value was larger than 0.9 (Fig. 2), indicating a continuous dominance of fungal residues. This might be the reason that the soils with a higher organic substrate preferentially retained and accumulated fungal hyphae (Guggenberger et al., 1999). Moreover, some previous reports also proposed that bacterial-derived MurN is less stable and decomposes more quickly than GluN produced by fungi (Solomon et al., 2002). On the other hand, the mineralization rate of bacterial residues is higher than that of fungal residues (Zhang et al., 2010). Compared with bacteria, fungi can use many resistant substances as energy sources (Bahram et al., 2018). As a result, fungi contribute more residues to the overall MRC because of their competitive advantage after afforestation.

4.3 Microbial residue accumulation and its contribution to SOC

Figure 3d–f shows a higher contribution of microbial residues to SOC in CL16 compared with MP16. This may be related to the larger amounts of resistant plant remnants in Mongolian pine plantations, such as lignin (Cotrufo et al., 2019). Furthermore, the application of fertilizer in cropland can promote microbial activities (Usmani et al., 2019), and microbial residues in young forests are easily decomposed and utilized by microorganisms without protection from clay particles (Khan et al., 2016). In both CL16 and MP16, FRC was consistently higher than BRC, indicating that the contribution of fungi to soil organic matter is greater than that of bacteria. This result can be the reason that fungal biomass is higher than that of bacteria in most ecosystems, and fungi are more effective than bacteria when utilizing the high C/N substrate (Bailey et al., 2002; Wang et al., 2021). In addition, the chemical resistance of fungal non-living biomass, especially cell wall constituents and pigments, causes a fungi-dominated ecosystem to stabilize more C than a bacteria-dominated population (Throckmorton et al., 2012).

In Mongolian pine plantation, the measured total MRC accounted for 29%–37% of SOC (Fig. 3), which suggests that microbial residues could serve as an immediate path for SOC accumulation in forests (Shao et al., 2019). There were no significant differences in MRC/SOC, BRC/SOC, and FRC/SOC ratios among Mongolian pine stands with different ages, which suggests that the contribution of microbial residues to SOC remained relatively steady (Li et al., 2022). This result may be related to the concurrent increase in the concentration of total and plant-derived SOC (Campo et al., 2019). Figure 3 shows that the concentrations of MRC, FRC, and BRC increase significantly with increasing stand age, which is consistent with previous observations (Maillard et al., 2021). Increased microbial activity in topsoil is the primary driver influencing the accumulation of MRC (Sokol et al., 2019). Therefore, as the years of afforestation increases, higher microbial biomass production and more rapid turnover cause more microbial

residues to be continuously retained in top soils (Spohn et al., 2016; Liang et al., 2017). Alternatively, the increased substrate and nutrients from the addition of litter after afforestation may reduce the rate of microbial mortality (Wang et al., 2021), finally leading to increased MRC, FRC, and BRC with increasing stand age.

5 Conclusions

This study evaluated changes in SOC and microbial residues following land use changes in the Horqin Sandy Land, China. After the establishment of Mongolian pine plantation on previous sandy land, SOC accumulation increased with stand age, and the phenomenon of soil acidification became increasingly apparent. Amino sugar stabilization became more effective under low soil pH condition after afforestation.

Increased plantation age also promoted the accumulation of amino sugars and MRC. In contrast to cropland, Mongolian pine plantation accumulated less SOC and MRC at the early stage. Across the five land-use patterns, calculated microbial parameters indicated that fungi, rather than bacteria, were consistently the main contributors to soil organic matter. Moreover, the contribution ratios of bacterial or fungal residues to SOC among Mongolian pine stands remained almost unchanged over time. We found that the duration of afforestation in the present research (16–62 a) was insufficient to disrupt the protection mechanisms of bulk organic C and influence the structure of microbial community. Our findings suggest that afforestation promoted positive compositional changes in soil organic matter by increasing microbial residues, which increases the persistence of SOC in Mongolian pine plantation.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Conceptualization: LU Sen, DU Zhangliu; Investigation: GUO Jingwen, SONG Xueshu; Methodology: LU Sen, DU Zhangliu; Formal analysis: GUO Jingwen, WANG Xiao; Writing - original draft preparation: GUO Jingwen; Writing - review and editing: LU Sen, GUO Jingwen; Funding acquisition: LU Sen. All authors approved the manuscript.

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